ATTACHMENT A

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:	)	
	)	
PAUL A. LUCIW, ET AL.	)	Group Art Unit: 1813
	)	
Serial No. 08/089,407	)	Examiner: M. Woodward
	)	
Filed: July 8, 1993	)	Attorney Docket No. 0035.008

For: HIV IMMUNOASSAYS USING SYNTHETIC ENVELOPE POLYPEPTIDES (AS AMENDED)

DECLARATION

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

I, John A.T. Young, do hereby declare as follows:

1. I received my Ph.D. in Human Genetics from Imperial Cancer Research Fund and University College, London, United Kingdom in 1987 having previously received a B.S. in Biochemistry from the University of Dundee in 1983.

2. I am currently an Assistant Professor, Department of Microbiology and Molecular Genetics, Harvard Medical School. My Curriculum Vitae is attached as Exhibit 1.

3. I have read and understand Luciw et al. application Serial No. 08/089,407 and Luciw et al. application Serial No. 08/667,501 ('501) as well as the Office Action mailed January 23, 1996.

4. One of ordinary skill in the art in 1984 understood the term "synthetic peptide" to mean a peptide prepared by chemical synthesis. The term "synthetic" was used to describe a peptide synthesized by chemical means in numerous publications prior to the October 31, 1984 filing date of parent application Serial No. 06/667,501. Representative publications (there are still others) include Altman 1984, Berkes 1984, Bellet 1984, Dale 1983, Green 1983, Hintz 1983, Hirayama 1982, Jacob 1983, Jolivet 1983, Lieu 1975, Morrow 1983, Morrow 1984, Muller 1983, Pacella 1983, Rothbard 1984, Rougon 1984, Sherwood 1983, Shi 1984, Sutcliffe 1983, Tamura 1982, and Wabuke-Bunod 1984.[1] The articles were published in a variety of well-known journals, including those read by a general scientific audience (e.g., *PNAS* and *Science*) as well as those read mainly by virologists and immunologists (e.g., *Journal of Virology and Molecular Immunology*). These are the journals that one skilled in the art would be expected to review.

5. Following 1984, the term "synthetic" was still understood by those skilled in the art to mean a peptide synthesized by chemical means. This is illustrated by the following sentence taken from Chapter 5 under the sub-heading "Synthetic peptides" of a widely-circulated laboratory research manual (Harlow, E., and D. Lane. 1988, Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.): "Peptides are normally synthesized using the solid-phase techniques pioneered by Merrifield (1963)." The term is still so-understood today.

[1]The full citation for each of the references cited in this declaration is included in Exhibit 2.

6. The prior art was capable of making a clear distinction between a synthetic peptide (i.e. one synthesized by chemical means) and a peptide fragment generated by some other means. See, Dale 1983, Hirayama 1982, Liau 1975, Morrow 1983, Morrow 1984, Muller 1983, Rothbard 1984, and Sherwood 1983.

7. Prior to October 31, 1984 one skilled in the art was fully capable of synthesizing peptides of considerable length. Specific examples of synthetic polypeptides containing as many as 40 amino acids were reported in the art prior to October 31, 1984. Ten of the above-mentioned articles (Altman 1984, Barkas 1984, Dale 1983, Hirayama 1982, Jacob 1983, Muller 1983, Rothbard 1984, Shi 1984, and Wabuke-Bunoti 1984) report synthetic peptides (i.e.: peptides made by chemical synthesis) having lengths of from 15 to 24 amino acids and one article (Bellet 1984) reports a 37 amino acid synthetic peptide. Reid (1981) employed a 34 amino acid synthetic peptide, while Puett (1982) employed a 40 amino acid synthetic peptide.

8. Immunoassays employing synthetic peptides such as claimed in the subject application were known in the art in 1984. Those techniques included ELISA analyses which employed peptides immobilized on microtiter plates, test sera, and enzyme-coupled secondary antibodies (e.g. Altman 1984, Bellet 1984, Green 1983, Jolivet 1983, Rothbard 1984, Wabuke-Bunoti 1984). Those techniques also included solid-phase radioimmunoassays that employed immobilized synthetic peptides, test sera, and ^{125}I-labeled protein A (Jacob 1983, Morrow 1984, Pacella 1983, Rothbard 1984,). Other methods were also known in the art in 1984 for detecting specific interactions between synthetic peptides and antibodies including radioimmunoassays that employed

radioactively-labeled peptides or antibodies (e.g. Barkas 1984, Hlintz 1982, Rougon 1984, Shi 1984, Tamura 1982).

9. The statement at page 3 of the '501 specification that "synthetic peptides may also be prepared" would have been understood by one of ordinary skill in the art in October 1984 as a teaching that such synthetic peptides would be used in the immunoassays described in the '501 specification. The '501 specification at pages 11, 14 and 15 specifically teaches that one use for the polypeptides of the invention is as antigens in a variety of immunoassays. One skilled in the art would not infer from the teaching of the patent specification that production of synthetic peptides would be a teaching of a useless act. One skilled in the art would be led to use the synthetic peptides in immunoassays just as the specification teaches.

10. The HIV nucleotide and amino acid sequences provided in the '501 parent application enabled one of ordinary skill in the art in October 1984 to identify synthetic HIV antigenic peptides, i.e., peptides containing an immunogenic amino acid sequence. To demonstrate this, I performed a hydrophilicity analysis of the ARV-2 Env sequence, according to the Hopp protocol (Hopp 1981, Hopp 1983). The directions in Hopp, together with the hydrophilicity values given in Hopp 1981, permit a straightforward analysis that was easily within the skill of the art in October 1984. The confirmation of antigenicity was also within the skill of the art in 1984. An antigen could be screened by using it in an immunoassay such as the prior art immunoassays identified in Paragraph 8 and testing it with sera of patients known to be infected. This screening process is the technique that is, in fact, disclosed in the Hopp references.

11. Employing the Hopp protocol, the most hydrophilic region of ARV-2 Env, was identified as residues 738-743 (ERDRDR). Synthetic peptides derived from HIV Env that contain these amino acid residues are recognized by a proportion of AIDS patient antisera as demonstrated by later actual tests. (Brolden 1992, Goudsmidt 1990, Kennedy 1986). The second-most hydrophilic region was identified as residues 653-658 (EKNEQE). Synthetic peptides containing this region of HIV Env are also recognized by sera from HIV infected individuals (Brolden 1992, Goudsmit 1990, Krowka 1991). The third most hydrophilic region of ARV-2 Env, residues 733-738 (EEEGGE), overlaps the first hydrophilic region. Synthetic peptides containing this third region of HIV Env are recognized by sera from HIV infected individuals. (Brolden 1992, Goudsmidt 1990, Kennedy 1986). The region containing residues 505-510 (QREKRA) was also identified as being highly hydrophilic. This finding was noted using the same computer analysis by Pauletti (1985). Synthetic peptides derived from HIV Env containing all or most of these residues are recognized by AIDS patient antisera (Brolden 1992, Kennedy 1987, Krowka 1991, Meshcheryakova 1993, Palker 1987, Streckert 1992).

12. Employing the Hopp protocol, the most hydrophilic region of ARV-2 Gag, was identified as residues 102-107 (EKEEEE). Synthetic peptides derived from HIV Gag that contain these amino acid residues are recognized by a proportion of AIDS patient antisera as demonstrated by later actual tests. (Jiang 1992). The second-most hydrophilic region was identified as residues 109-114 (NKSKQQ). Synthetic peptides containing this region of HIV Gag are immunogenic and are recognized by sera from HIV infected individuals (Jiang 1992).

13. The HIV sequences provided in the '601 parent application also enabled one of ordinary skill in the art in October, 1984 to identify antigenic HIV Env linear epitopes by still other techniques. One other approach known in the art, was to generate one or a panel of several synthetic peptides derived from the polypeptide sequence and test each peptide for antibody reactivity. The generation of one or a panel of synthetic polypeptides from a single protein was a routine matter in 1984.

14. A panel of eight peptides (each 13-15 amino acids in length) of interleukin-2 was generated by Altman (Altman 1984) and a panel of five synthetic peptides (8 to 16 amino acids long) derived from adenovirus 19K and 53K proteins was generated by Green. (Green 1983). In addition, Sutcliffe generated a panel of 12 peptides from MuLV polymerase gene and a panel of 18 peptides from the rabies glycoprotein gene. (Sutcliffe 1983)

15. Prior to October 1984, those skilled in the art knew that a proportion of antibodies raised against native proteins could recognize epitopes contained on synthetic peptides derived from a protein sequence (Rainbird 1984; Leach 1983) or contained on proteolytic protein fragments (Lerner 1982).

16. Based on the information described herein, those skilled in the art could have, without undue experimentation, used the sequence of ARV-2 Env provided in the '501 application to generate synthetic peptides representing most of the HIV glycoprotein. These peptides could then have been tested using standard assays known in the art, and immunogenic regions of HIV Env identified.

17. I have reviewed in detail Montagnier, *Science*, 225, 63-66 (July, 1984) and Schupbach et al., *Science*, 224, 503-505 (May, 1984). In my opinion these articles would not have enabled one skilled in the art to prepare a syntheric HIV envelope polypeptide sequence for use in an immunoassay without undue experimentation. I conclude this for the following reasons:

a) These articles did not provide any HIV nucleotide or amino acid sequence information.

b) Although HIV proteins were purportedly identified by immunoblotting in these publications, a person of ordinary skill in the art would not have been able to produce sufficient quantities of any of these viral proteins for sequencing. Sufficient quantities could not have been produced because cultures of primary human cells failed to produce significant quantities of HIV, as the virus is cytopathic and rapidly killed the infected virus-producing cells. Therefore, a person of ordinary skill in the art, attempting to generate sufficient quantities of HIV proteins for detailed characterization, would have i) had to obtain an appropriate established cell line known to produce HIV and ii) had to have a knowledge of the precise conditions required for infecting these cells and for maintaining the infected cells for long periods of time in culture.

c) By October 31, 1984, the Gallo and Montagnier groups had reported cell lines that could be used to produce significant levels of HIV (Popovic 1984, Montagnier 1984). Gallo and Montagnier were world

leaders in HIV research at the time, and thus can hardly be considered to be of "ordinary skill in the art". At the time of the '501 application date, the precise origin of the cell line used by the Gallo group had not been disclosed (Popovic 1984). The Montagnier group used cells generated by fusion between HIV producing primary T cells and EBV-transformed B-cells (Montagnier 1984). It would not have been possible for a scientist of ordinary skill in the art to have used the same technique to produce cell lines that were identical to those described by the Montagnier group. Even if a scientist of ordinary skill in the art had attempted to obtain the cells described by the Gallo and Montagnier groups, I am not aware of any evidence that these cell lines were being distributed freely to the public at the time of the '501 application date. Furthermore, the precise culture conditions required for maintaining HIV-infected cells in culture had not been disclosed.

18. The announcement by the Gallo group that HTLV-III was related to HTLV-I and II, such as contained in Gallo et al. (1983) and Arya et al. (1984), led workers such as Chang to incorrectly presume that the Env gene was located at the same position in the HIV and HTLV-I and II genomes. Furthermore, the Gallo group proposed that the HIV genome contains a pX or LOR region similar to those found in HTLV-I and II. In fact, as the '501 application correctly disclosed, a) HIV is not closely related to HTLVs, b) the Env gene is not located at the same position in the HIV and HTLV genomes and c) there is no pX or LOR region in the HIV genome.

19. The presumption that HIV was closely related to HTLV-I and ll led the Gallo group to seriously misidentify HIV envelope proteins:

a) The Gallo group described a 65 kD HIV protein as "envelope-related" apparently because it migrated on SDS-polyacrylamide gels at a position similar to that of the 62-65 kD HTLV precursor envelope protein (Schupbach 1984). The HIV precursor envelope protein is, however, a 160kD protein (designated gp160), a fact that only came to light after the '501 application filing date.

b) The Gallo group described a 41 kD HIV protein as "the presumed envelope antigen of the virus" (Samgadharan 1984). The 41 kD protein was shown to be an antigenic viral structural protein (Samgadharan 1984). However, the inescapable conclusion from this manuscript was that these workers presumed that this viral protein was envelope-related because it was similar in size to the 46kD HTLV envelope protein (gp46; Samgadharan 1984) i.e., the HIV p41 protein was equivalent to HTLV gp46. In fact, these proteins are not equivalent for the following reasons:

i) All retroviral envelope proteins are synthesized as precursor proteins (see ¶19a) that are cleaved into two mature subunits designated surface (SU) and transmembrane (TM). These two envelope proteins remain associated together after this cleavage and are incorporated together onto the surface of viral particles.

However, these proteins are structurally distinct and perform different functions during viral entry; the SU protein is primarily involved in receptor binding, whereas the TM protein contains the transmembrane region that anchors the envelope proteins on the virus surface. The TM protein is primarily involved at a step of viral entry following receptor binding.

ii) The SU and TM proteins of HIV are designated gp120 and gp41 (the 41 kD protein described by Samgadharan 1984), respectively. The SU (gp120) protein of HIV was not described prior to the '501 application filing date.

iii) The SU protein of the HTLVs is gp46 and the TM protein of the HTLVs is p20E, a 20 kD protein.

20. I have also reviewed in detail Chang U.S. application Serial No. 659,339 filed October 10, 1984 including the partial DNA sequence of Figure 3. The Chang specification (1) incorrectly describes the location of the Env gene in the HIV genome, and (2) misrepresents the sequence of the Env gene which is purported to be encompassed (i.e. wholly-contained) within the DNA sequence shown in Figure 3. An individual skilled in the art attempting to identify the HIV Env open reading frame found in the sequence of Figure 3 would have been unable to do so.

21. Although Chang represents that the Figure 3 sequence "encompasses the *env* region" (p. 5, lines 1-2), that is incorrect. In fact, the Figure 3 sequence contains a

portion of the *pol* gene, the *sor* gene and only approximately one-third of the envelope gene.

22. Moreover, the Chang Figure 3 sequence includes an error. The Figure 3 sequence includes an extra nucleotide ("A") at position 2497, a residue which does not actually exist in the HIV envelope gene. This mistake leads to a +1 translational frameshift at this position in the partial sequence of the envelope open reading frame. As a consequence of this error, this open reading frame is only correct over the region encoding the first 63 amino acids of Env (including the N-terminal signal peptide which is removed during protein biosynthesis). The open reading frame of the Figure 3 sequence then continues with three amino acids encoded by an incorrect reading frame followed by a stop codon.

23. Based on Figures 1 and 2 of Chang, a scientist would have been completely misled about the placement of the envelope gene relative to restriction enzyme sites in the HIV genome, e.g., an EcoR1 site that is actually located upstream of the envelope gene is shown in the Chang '339 application both as contained within the envelope gene (Figure 1) and upstream of the envelope gene (Figure 2). Also, a Bgl II site, which is actually located in the envelope gene, is shown in the Chang application as within the "pX" region, a region which does not exist in the HIV genome. HIV is not closely related to HTLV-I and II, and unlike these other human retroviruses HIV certainly does not contain a pX region.

24. Based, *inter alia*, on the above-identified defects, Chang did not enable one skilled in the art in October, 1984 to grow, isolate and/or sequence the envelope gene of HIV.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: March 19, 1997 By: _____
John A.T. Young